

JOURNAL

OF THE

AMERICAN CHEMICAL SOCIETY

(Registered in U. S. Patent Office)

CONTENTS

PHYSICAL CHEMISTRY

- | | | | |
|---|-----|---|-----|
| Ralph K. Birdwhistell and Ernest Griswold: The Effect of Some Salts on the Solubility of Silver Acetate and of Silver Nitrate in Acetic Acid at 30° | 873 | E. Lee Purlee, Robert W. Taft, Jr., and C. A. DeFazio: Enthalpies and Entropies of Activation for the Hydration of Dissolved Isobutene and Trimethylethylene from the Thermodynamic Properties for Solution of Gaseous Olefins in Aqueous Nitric Acid | 837 |
| R. K. Branton: The Photolysis of Acetaldazine | 842 | F. F. Rawlings and E. C. Lingafelter: X-Ray Crystallography of the Sodium <i>n</i> -Alkyl Sulfates | 870 |
| James P. Coughlin: High-Temperature Heat Contents, Heat of Transition, and Heat of Fusion of Anhydrous Sodium Sulfate | 868 | Notes | |
| Isaac Feldman, T. Y. Toribara, Jean R. Havill and W. F. Neuman: The Beryllium-Citrate System. II. Ion-exchange Studies | 878 | Fred H. Coats and Robbin C. Anderson: Electron Impact Data on Substituted Acetylenes: Propyne and 2-Butyne | 895 |
| Paul Yen-hsiung Feng and Joseph W. Kennedy: Electrical and Chemical Effects of β -Radiation in Polystyrene | 847 | Walter Dannhauser and Philip A. Vaughan: The Crystal Structure of Cuprous Chromite | 896 |
| Harold Hart and William L. Spliethoff: Kinetics of the Racemization of α -Phenethyl Chloride in Phenols | 833 | O. J. Kleppa: The Heat of Formation of InSb | 897 |
| Norman C. Li, Ting Li Chu, Charles T. Fujii and James M. White: Association of Imidazole with Nickel(II) and Alkaline Earth Ions | 859 | H. R. Owen and O. A. Schaeffer: The Isotope Abundances of Chlorine from Various Sources | 898 |
| William F. Linke: The System Magnesium Bromate-Water | 866 | Ralph P. Seward: The Conductance and Viscosity of Highly Concentrated Aqueous Solutions of Hydrazinium Chloride and Hydrazinium Nitrate | 905 |
| R. S. Mulliken: Structures of the Halogen Molecules and the Strength of Single Bonds | 884 | Robert W. Taft, Jr., E. Lee Purlee and Peter Riesz: A Method for Determining the Distribution Constant for a Substance between the Gas Phase and a Condensed Phase | 899 |
| R. S. Mulliken: Bond Angles in Water-Type and Ammonia-Type Molecules and their Derivatives | 887 | Robert W. Taft, Jr., and Peter Riesz: Thermodynamic Properties for the System Isobutene- <i>n</i> -Butyl Alcohol | 902 |
| Robert T. O'Connor, Robert R. Mod, Mildred D. Murray and Evald L. Skau: The X-Ray Diffraction and Infrared Spectra of Molecular Compounds of Acetamide and Long-Chain Saturated Fatty Acids | 892 | J. R. Tomlinson, L. Domash, R. G. Hay and C. W. Montgomery: The High Temperature Heat Content of Nickel Oxide | 900 |

INORGANIC CHEMISTRY

- | | | | |
|---|-----|--|-----|
| Gilbert H. Ayres and Max H. Booth: Catalytic Decomposition of Hypochlorite Solution by Iridium Compounds. I. The pH-Time Relationship | 825 | Volumes and Electrical Conductivities of the Molten System: Molybdenum Trioxide-Sodium Molybdate | 851 |
| Gilbert H. Ayres and Max H. Booth: Catalytic Decomposition of Hypochlorite Solution by Iridium Compounds. II. Kinetic Studies | 828 | Alexander I. Popov and Wesley W. Wendlandt: The Methylamine Complexes of the Rare Earth (III) Chlorides | 857 |
| William V. Hough, George W. Schaeffer, Marcelline Drurus and Albert C. Stewart: The Preparation, Identification and Characterization of the <i>N</i> -Trialkylborazoles | 864 | Walter C. Schumb and Walter J. Bernard: The Thiochlorides of Silicon | 862 |
| E. H. Huffman, G. M. Iddings, R. N. Osborne and G. V. Shalimoff: Extraction of Zirconium and Hafnium with Various Fluorinated β -Diketones | 881 | A. K. Sundaram and E. B. Sandell: Chloro Complexes of Palladium(II) in Solution | 855 |
| M. H. Lietzke and J. V. Vaughan: The Behavior of the Silver, Silver Chloride and the Mercury, Mercurous Chloride Electrodes at High Temperatures | 876 | Notes | |
| Kelso B. Morris, Marlene I. Cook, Clarice Z. Sykes and Malcolm B. Templeman: Densities, Molal | | Dietmar Seyferth and Eugene G. Rochow: The Preparation of Chloromethyl Derivatives of Germanium and Silicon by the Diazomethane Method | 907 |
| | | Walter C. Schumb and Walter J. Bernard: The Formation of Silicon Monosulfide | 904 |

($1-2 \times 10^5$ MSH u./g.) was obtained. Eight grams was mixed with 150 ml. of 0.1 *N* acetic acid and centrifuged. Sixteen grams oxycellulose was added to the supernate and the mixture shaken for 75 min. Oxycellulose was removed by centrifugation, washed with 0.1 *N* acetic acid, then shaken in 100 ml. of 80% acetic acid for 60 min. The supernate was diluted with equal quantities of water. Lyophilization yielded 0.35-0.5 g. product ($1-2 \times 10^5$ MSH u./g.). One and a half grams of this fraction was distributed through a 12-tube counter-current system at 5° using *sec*-BuOH and 0.5% aqueous trichloroacetic acid. The contents of tubes 4-6 were combined and lyophilized. Approximately 0.5 g. of solids ($3-4 \times 10^5$ MSH u./g.) was obtained. Forty mg. was subjected to paper electrophoresis at 5°, 18 volts/cm., 8-10 hours, pH 8.9 using barbiturate-acetate-hydrochloric acid buffer ($\mu = 0.056$). Four components were visualized with 1% brom phenol blue staining. That moving fastest toward the cathode was extracted with 20% acetic acid and lyophilized. The product was dissolved in 1 ml. of 0.2 *N* acetic acid and subjected to paper electrophoresis at pH 4.9 using pyridine-acetic acid buffer ($\mu = 0.1$), 5°, 18 volts/cm., 10-12 hours. Staining revealed a single component moving toward the cathode. The active area was extracted with 20% acetic acid and lyophilized. The white solid, 2.5 mg., ($1.5-2.5 \times 10^{10}$ MSH u./g.) represented about 30% of the total MSH activity placed on the first electrophoretic run at alkaline pH. Ninhydrin reaction of hydrolyzed extracts of different parts of the filter paper run at

pH 4.9 showed MSH activity associated with the predominant color response.

The active fraction moved as a single component (staining with bromophenolblue) on paper electrophoresis at pH 1.4, 4.9, 8.9, 11.3 and 12.2. Since at pH 11.3 movement towards the anode was slight compared with dextran, the iso-electric pH was estimated to be in the region of 10.5-11. On the basis of amino acid composition minimum molecular weight was estimated at 4500. MSH activity of the final product was approximately 500 times that of the original hog posterior pituitary powder with little ACTH activity.¹⁴ This fraction behaved as a single component when distributed in a 97 tube counter-current apparatus employing the solvents described previously. Although the MSH preparation, assumed to be a polypeptide, was tested by electrophoresis and counter-current distribution, other criteria for homogeneity remain to be satisfied.

(14) MSH has little if any vasopressin or ACTH activity. Assays set to detect one unit each of ACTH or vasopressin, using 378 and 449 mcg. of MSH, respectively showed no activity.

DIVISION OF DERMATOLOGY AARON B. LERNER
UNIVERSITY OF OREGON MEDICAL SCHOOL TEH H. LEE
PORTLAND 1, OREGON

RECEIVED JANUARY 13, 1955

A NEW METHOD OF FORMING PEPTIDE BONDS Sir:

We wish to describe a new and very useful method of forming peptide or other amide bonds. The two components, one containing a free carboxyl function and the other a free amino group, couple directly and rapidly in high yield on treatment with *N,N'*-dicyclohexylcarbodiimide at room temperature.

In contrast to other schemes for carboxyl activation involving mixed anhydride formation, the reaction is not sensitive to moisture; indeed, it may be carried out in aqueous solution. The remarkable selectivity of the reagent is attested by the successful use of carbobenzoxyserine as an acylating moiety without protection of the hydroxyl group. No racemization was detected employing as the acylating agent a dipeptide derivative in which an optically active amino acid furnished the free carboxyl function (carbobenzoxyglycyl-L-phenylalanine), an observation of considerable importance in the synthesis of larger peptides by joining units containing two, three or more amino acids. The co-product, *N,N'*-dicyclohexylurea, has a very low solubility in most organic or aqueous solvents, and, in all cases tried, is easily separated.



The simplicity, convenience and efficiency of this technique may be illustrated by the synthesis of a tripeptide derivative. After a 4-hour period at room temperature, a solution in tetrahydrofuran of carbobenzoxyglycyl-L-phenylalanine containing a slight excess of crystalline *N,N'*-dicyclohexylcarbodiimide¹ and ethyl glycinate was treated with a small amount of acetic acid (to decompose the

(1) Readily prepared by the method of R. Herbeck and M. Pezzati, *Ber.*, 71, 1033 (1938).

TABLE

Amino acid ^a	Per cent. ¹²	Molecular ratio ¹³
Aspartic	3.8	3
Glutamic	5.3	3
Serine	3.4	3
Glycine	2.3	3
Tyrosine	4.1	2
Lysine	5.3	3
Arginine	4.0	2
Valine	2.9	2
Phenylalanine	4.7	3
Alanine	1.4	1
Cystine ¹⁰	5.3	2
Proline	3.7	3
Leucine	1.7	1
Threonine	1.3	1
Histidine	0.6	0
Tryptophan ¹¹	...	2
Total	49.8	34

(9) Semi-quantitative amino acid analyses were done by A. M. Gross and W. F. White of the Research Department, Armour Laboratories using filter paper chromatography and determining the intensity of ninhydrin stained areas with a densitometer; J. F. Rowland and A. M. Gross, *Anal. Chem.*, 26, 502 (1954).

(10) Cystine and cysteine are not distinguished in the analysis. However, cysteine is probably absent because MSH is not oxidized and reduced readily as would be expected were this amino acid present. Methionine was not tested for.

(11) Tryptophan was determined by ultraviolet absorption after subtracting tyrosine from the total value; A. B. Lerner and C. F. Barnum, *Arch. Biochem.*, 10, 417 (1948).

(12) Tryptophan, methionine, moisture and ash were not included in the total amino acid per cent. analysis.

(13) Molecular ratios are given in whole numbers and represent only approximate values.

excess reagent). The insoluble urea was removed, the solvent was replaced by ethyl acetate, and the solution was washed with dilute acid and aqueous potassium bicarbonate. The addition of petroleum ether afforded 87% of crystalline carbobenzoxyglycyl-L-phenylalanylglycine ethyl ester; m.p. 118–119°, $[\alpha]_D^{25} -13.5^\circ$ [ethanol] (reported²: m.p. 116–118°, $[\alpha]_D^{25} -12^\circ$). In a similar fashion we have prepared a variety of dipeptide derivatives, including the following examples.

In methylene chloride, phthaloyl-L-phenylalanylglycine ethyl ester was produced in 92% yield; m.p. 161–162°, $[\alpha]_D^{25} -146^\circ$, (reported³: m.p. 161–162°, $[\alpha]_D^{25} -146^\circ$). In aqueous tetrahydrofuran, a product of the same quality was obtained in 72% yield. Phthaloyl-L-alanyl-L-proline benzyl ester (74%) was isolated with m.p. 101–102°, $[\alpha]_D^{25} -135^\circ$ [ethanol]. *Anal.* Calcd. for $C_{23}H_{25}N_2O_6$: C, 67.98; H, 5.42; N, 6.90. Found: C, 68.07; H, 5.52; N, 6.77. Carbobenzoxy-L-serine and ethyl glycinate coupled to give carbobenzoxy-L-serylglycine ethyl ester (59%) in tetrahydrofuran: m.p. 106–107°, [ethanol], reported⁴ m.p. 105–107°. Phthaloyl-L-phenylalanyl-L-leucine ethyl ester (91% yield) had a m.p. of 109–110°, $[\alpha]_D^{25} -115^\circ$ [ethanol]. *Anal.* Calcd. for $C_{23}H_{25}N_2O_6$: C, 68.78; H, 6.47; N, 6.42. Found: C, 68.50; H, 6.59; N, 6.48.

DEPARTMENT OF CHEMISTRY JOHN C. SHEEHAN
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS GEORGE P. HESS⁵

RECEIVED JANUARY 11, 1955

(2) G. Anderson and R. Young, *THIS JOURNAL*, **74**, 5307 (1952).

(3) J. Sheehan, D. Chapman and R. Roth, *ibid.*, **74**, 3822 (1952).

(4) J. S. Fruton, *J. Biol. Chem.*, **145**, 463 (1942).

(5) Aided by a fellowship from the National Foundation for Infantile Paralysis.

9 α -HALO-11 β -HYDROXY AND 11-KETO DERIVATIVES OF PROGESTERONE, DESOXYCORTICOSTERONE AND 17 α -HYDROXYPROGESTERONE

Sir:

In previous communications^{1,2} there have been described the synthesis of 9 α -halogenated derivatives of cortisone and hydrocortisone and shown that the glucocorticoid activity of these substances increased with decreasing atomic weight of the halogen atom. The most active member of that series, 9 α -fluorohydrocortisone acetate possessed about 11 times the activity of cortisone acetate in the rat liver glycogen assay. Soon thereafter it was found that in addition to being potent glucocorticoids these compounds were highly effective in controlling electrolyte balance and in maintaining life in the rat³, dog^{4,5} and in man.^{4,6}

It appeared of great interest to ascertain what influence variations in the side-chain might have upon the adrenocorticoid activity of such halogen-

ated derivatives. For this purpose we have prepared the 9 α -halo derivatives (halogen = Br, Cl, F) of 11 β -hydroxyprogesterone, 11 β ,17 α -dihydroxyprogesterone and corticosterone acetate and of the corresponding 11-ketones by a synthetic route paralleling that described in our earlier publications.^{1,2} This synthesis proceeds from the 11-mesylates of the requisite 11 α -hydroxy derivatives⁷ (11 α -hydroxyprogesterone mesylate, m.p. 165–167°; $[\alpha]_D^{25} +135^\circ$ (c, 0.77 in $CHCl_3$); λ_{max}^{alc} 238 m μ ($\epsilon = 17,200$); *Anal.* C, 64.81; H, 7.63; S, 7.48. Epicorticosterone 11 α -mesylate 21-acetate, m.p. 156–157°; $[\alpha]_D^{25} +144^\circ$ (c, 0.92 in $CHCl_3$); λ_{max}^{alc} 238 m μ ($\epsilon = 16,600$); *Anal.* C, 61.52; H, 7.07. 11 α ,17 α -Dihydroxyprogesterone 11 α -mesylate, m.p. 150–152°; $[\alpha]_D^{25} +64^\circ$ (c, 0.49 in $CHCl_3$); λ_{max}^{alc} 238 m μ ($\epsilon = 18,200$); *Anal.* C, 62.11; H, 7.71; S, 7.11), via the 9,11-unsaturated steroids (9(11)-dehydro-17 α -hydroxyprogesterone, m.p. 214–216°; $[\alpha]_D^{25} +67^\circ$ (c, 0.82 in $CHCl_3$); λ_{max}^{alc} 239 m μ ($\epsilon = 18,450$); *Anal.* C, 76.52; H, 8.46), to the 9 α ,11 β -bromohydrins (see table). The latter on treatment with base yielded the 9 β ,11 β -epoxides (9 β ,11 β -oxidoprogesterone, amorphous, $[\alpha]_D^{25} +61^\circ$ (c, 1.55 in $CHCl_3$); λ_{max}^{alc} 243 m μ (ϵ 13,600). 9 β ,11 β -Oxidodesoxycorticosterone acetate, m.p. 137–138°; $[\alpha]_D^{25} +61^\circ$ (c, 0.66 in $CHCl_3$); λ_{max}^{alc} 243 m μ ($\epsilon = 15,100$); *Anal.* C, 71.81; H, 8.10. 9 β ,11 β -Oxido-17 α -hydroxyprogesterone, m.p. 183–184°; $[\alpha]_D^{25} -32^\circ$ (c, 1.02 in $CHCl_3$); λ_{max}^{alc} 243 m μ ($\epsilon = 16,600$); *Anal.* C, 72.99; H, 8.11), which upon reaction with the requisite hydrogen halides formed the 9 α -chloro- and 9 α -fluoro-11 β -hydroxy derivatives. Oxidation with chromic acid furnished the corresponding 11-ketones. Alternatively, the 9 α -chloro derivatives could be prepared by allowing the 9(11)-unsaturated steroids to react with N,N'-dichlorodimethylhydantoin in the presence of perchloric acid.⁸

The physical properties of the halogenated steroids and the activities of representative compounds in the liver glycogen and sodium retention assays in the adrenalectomized rat are listed in the accompanying table. As had been observed previously in the 9 α -halohydrocortisone series both gluco- and mineralocorticoid activities were found to increase with decreasing atomic weight of the halogen atom. No significant differences were noted between the activities of the 11 β -hydroxy and 11-keto derivatives. Outstanding among the compounds tested were 9 α -fluoro-11 β -hydroxy and 11-ketoprogesterone, which although lacking both the 17- and 21-hydroxyl groups approximately equalled cortisone acetate in glucocorticoid activity. The most potent mineralocorticoids of this series were 9 α -fluorocorticosterone acetate and 9 α -

(1) J. Fried and E. F. Sabo, *THIS JOURNAL*, **75**, 2273 (1953).

(2) J. Fried and E. F. Sabo, *ibid.*, **76**, 1456 (1954).

(3) A. Borman, F. M. Singer and P. Numerof, *Proc. Soc. Exp. Biol. Med.*, **86**, 570 (1954).

(4) G. W. Liddle, M. M. Pechet and F. C. Bartter, *Science*, **120**, 498 (1954).

(5) W. W. Swingle, C. Baker, M. Eisler, S. J. Le Brie and L. J. Brannick, *Proc. Soc. Exp. Biol. Med.*, in press.

(6) A. Goldfin, G. W. Thorn, P. M. Beigleman and J. C. Laidlaw, *J. Clin. Endocrinology*, **14**, 782 (1954).

(7) J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Doniu and D. Perlman, *THIS JOURNAL*, **74**, 3982 (1952).

(8) The course of this reaction was dependent on the nature of the side chain. Thus, 9(11)-dehydro-17 α -hydroxyprogesterone afforded the desired chlorohydrin in about 60% yield. On the other hand, treatment of 9(11)-dehydroprogesterone with N,N'-dichlorohydantoin resulted in a mixture containing more than one atom equivalent of chlorine from which 9 α -chloro-11 β -hydroxyprogesterone could be isolated only after reduction with chromous chloride. It appears likely that the extra chlorine atom reducible by chromous chloride is located in the 17-position.